REMARKS

Affirmation of Prior Election of Invention

Applicants hereby cancel claims 3-8, 11, 13-22, 26 and 30-32 in response to the imposed restriction requirement with the right to file divisional application on any and all cancelled subject matter.

Rejection of Claims and Traversal Thereof

In the August 11, 2003 Office Action:

claims 1-2, 4, 9, 12, 23-24 and 27-28 were rejected under 35 U.S.C. §112, first paragraph; and

claims 12 and 27 were rejected under 35 U.S.C. §102(a) and (b) as being anticipated by Seki, et al. 1998, Saito, T. et al. 1999, or Naik, M. U., et al. 1999, Genbank Sequence Database (Accession No: Q9Z0F4).

These rejections are hereby traversed and reconsideration of patentability of the pending claims is therefore requested in light of the following remarks.

Rejection under 35 U.S.C. §112, first paragraph

In the August 11, 2003 Office Action, claims 1-2, 4, 9, 12, 23-24 and 27-28 were rejected under 35 U.S.C. §112, first paragraph. The only issue raised in the August 11, 2003 Office Action is one of enablement. This rejection is hereby traversed and reconsideration of the patentability of the pending claims, as amended herein, is requested in light of the following remarks.

According to the Office:

> "[I]t is unpredictable that reduction in apoptosis, in Hela cells transfected with the mutant EF-N of SEQ ID NO.: 2 and PS2 of SEQ ID NO: 1, as compared to apoptosis caused by PS2 of SEQ ID NO: 1 alone is caused by inhibition of the interaction of SEQ ID NO: 1 and said mutant."

Applicants note that, as described in the specification at page 30, there is definite proof that there is binding between PS2 and calmyrin. The specification discusses the fact that when calmyrin was

coexpressed with PS2, calmyrin's staining pattern was dramatically altered such that it colocalized almost completely with PS2 (Figs. 22-23). As exemplified by the two cells shown in panels A and B, the calmyrin protein was less apparent in the nucleus in coexpressing cells than in cells transfected solely with calmyrin (Fig. 14). Another indication that these two proteins bind each other was seen in a small subset of cells where calmyrin and PS2 colocalized distinctively in unusual intranuclear spots (Fig. 24). The shift in calmyrin localization and the nearly identical staining patterns between PS2 and calmyrin (see merged images) in these coexpressing cells provide persuasive evidence that these two proteins interact *in vivo*.

Furthermore, when calmyrin was cotransfected with a PS2 construct deleted of the loop and all sequence COOH-terminal of it, the staining patterns displayed significantly less overlap; as seen by patchy aggregates of PS2 which excluded calmyrin (Fig. 25, indicated by arrows). The failure of this PS2 deletion construct to completely colocalize with calmyrin in aggregates, which contrasts with the colocalization of the wild-type PS2 protein and calmyrin in nuclear inclusions, enhances the belief that the PS2-loop region facilitates binding of calmyrin. Thus, it has been shown in the present application that PS2 interacts with the calcium-binding protein calmyrin and that the structural features of calmyrin and presenilins implicated apoptotic signaling and Ca^{2+} regulation as playing potential roles in the PS2/calmyrin interaction. As such, applicants determined that reduced Ca^{2+} affinity EF-hand mutants of the calmyrin protein would reduce interaction between PS2 and calmyrin.

As described at pages 36 and 37 of the present specification, mutations in the two Ca^{2+} -binding EF-hands were created by the QuikChange site-directed mutagenesis method (Stratagene) using the pBS-calmyrin construct with PCR primers EF(N)l (SEQ ID NO: 21) and 2 (SEQ ID NO: 22) or EF(C)l (SEQ ID NO: 23) and 2 (SEQ ID NO: 24) to generate, respectively, the D127N mutation by changing the first base in the codon from G to A and the E172Q mutation by changing the first base in the codon from G to C. Specifically, in the calmyrin-EF-N mutant the aspartic acid at position 127 of protein SEQ ID NO: 2 in the first intact EF-hand in calmyrin was mutated to asparagines (G to A at position 445 of nucleotide SEQ ID NO: 26) . Similarly, the calmyrin-EF-C mutant had the glutamic acid at position 172 of protein SEQ ID NO: 2 in the second intact EF-hand mutated to glutamine (G to C at position 584 of nucleotide SEQ ID NO: 26). The acidic residues were replaced with their amine counterparts so that the affinity for Ca^{2+} would be lowered while maintaining structural integrity.

According to the Office:

> "Applicant has not shown that the calcium binding domain is necessary for the binding and interaction with PS2 of SEQ ID NO:1."

Applicants strongly disagree. As stated above, the structural features of calmyrin and presenilins implicated apoptotic signaling and Ca^{2+} regulation as playing potential roles in the PS2/calmyrin interaction and it was shown that reduced Ca^{2+} affinity EF-hand mutants of the calmyrin protein reduced interaction between PS2 and calmyrin.

As shown and discussed at page 39 of the present specification, coexpression of wild type calmyrin with PS2 increased cell death additively (seen in both Fig. 39 and Fig. 40), coexpression of each of the three calmyrin mutants **with PS2 decreased cell death below the 2.09 level seen with PS2 alone**. When compared to PS2, the decrease in cell death from the coexpression of the EF-hand mutants reached statistically significance, EF-C mean=l .05 (p-value=0.006) and EF-N mean=l .00 (p-value=0.005). It is especially remarkable that cell death returned to control levels with the coexpression of PS2 and EF-C, two proteins that individually increase cell death 2.1 fold and 1.6 fold respectively. **This unanticipated result suggests that the mutant calmyrin interferes with PS2 induced cell death, further indicating that these two proteins interact in a functionally significant manner. Clearly, the calcium-binding domain is essential for binding and interaction and the results above indicate that a mutation in this specific area greatly reduces binding and interaction and thus reduce apoptosis.**

The Office has faulted the applicants for not providing *in vivo* examples to show the efficacy of the claimed invention in human subjects. Applicants submit that the efficacy of the compositions of this invention is fully and rigorously established by applicants' empirical determinations applicants tested the efficacy of the claimed compositions *in vitro* to determine the positive results showing reduces apoptosis. Further, applicants remind the Office that the standard for enablement and thus patentability is not the same as that required for drug marketing approval by the Federal Drug Administration. See *Scott v. Finney*, 32 USPQ2d 115 (Fed. Cir. 1994). Patentability and enablement do not hinge on the outcome of human clinical trials.

Applicants insist that to be enabling, the specification must simply set forth "a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same. Applicants have met this standard.

Applicants reiterate Hela cells have been used in studies investigating drugs and other compounds because there is a clear correlation between results found *in vitro* testing using Hela cells and *in vivo* testing models. Applicants have provided multiple examples indicating a positive correlation between *in vitro* results found in Hela cells and *in vivo* results.

The references cited by the Office discuss cell cultures but a primary or secondary cell culture cannot be compared to immortalized cells such as Hela cells which unlike primary or secondary cells, continue to grow and divide indefinitely *in vitro* for as long as the correct culture conditions are maintained. Hela cells are the classic example of an immortalized cell line and are adherent cells and not oncogenic in animals, unless transformed by a virus. Specifically, the Drexler, et al. reference, cited by the Office, states at page 3, that the Hodgkin's disease HD cell lines used in the studies were not immortalized cells, and thus, cannot be compared to immortalized Hela cells. Embleton, et al., also cited by the Office, discusses the lack of antigens on cultured cells thereby reducing the accuracy of interpreting results obtained with monoclonal antibodies but the present invention does not include the production of antibodies for the Hela cells or tumor cells but instead antibodies are raised for epitopes on calmyrin. Thus, the Embleton, et al reference is not relevant to the presently claimed invention. The Office further cites Freshney that discusses the disadvantages of *in vitro* cell cultures and states that cellular metabolism may be more constant in *in vitro* than *in vivo*. However, it must be recognized by the Office that Freshney further states that any inconsistency in the cell cultures can be rectified by inclusion of a number of different hormones in the culture media.

Hsu, also cited by the Office, discusses different analysis for monitoring cell population by the chromosome constitution of the *in vitro* cell line. However, applicants do not even consider the chromosome constitution of the cultured cells as being relevant to the present invention, and instead, as clearly stated in the present application, monitor the level of apoptosis by a simple procedure that can include manual counting of dead cells. Clearly, none of the references cited by the Office provide any definitive proof that *in vitro* and *in vivo* test results are not comparable. Likewise, the Office has provided no proof that the overexpression of calmyrin, mutated calmyrin or presenilin altered the cultured cells or its colonization behavior.

According to the Office, "

> "Applicants only show that a single conservative substitution at amino acid 127 reduces the apoptosis induced by PS2, which cannot be extrapolated to substitutions at any amino acids in the calcium binding EF hand of calmyrin ... because the effect of these substitution on apoptosis are unpredictable."

Applicants have provided sufficient disclosure, either through illustrative examples or terminology, to teach those of ordinary skill how to make and use the invention by discussing the exact regions for mutations in the calmyrin protein that have been found effective to reduce binding affinity with the presenilins. Specifically, as stated above, these regions include: the calcium-binding EF-hands of SEQ ID NO: 2, including amino acid residues at positions 116 to 128 and 161 to 173; and the N-terminal of SEQ ID NO: 2. Furthermore, applicants have shown the effectiveness of replacing acidic residues with their amine counterparts so that the affinity for Ca^{2+} would be lowered thereby reducing affinity for PS2 and reducing apoptosis.

It is well settled in the law that a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of Section 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. The Office has not provided any evidence that one of ordinary skill in the art would doubt the objective truth of the statements contained in applicants' disclosure, and thus, all claims as now amended meet the requirements under 35 U.S.C. §112, first paragraph, and withdrawal of the rejections is respectfully requested.

Rejection under 35 U.S.C. §102(a) and (b)

Claims 12 and 27 were rejected under 35 U.S.C. §102(a) and (b) as being anticipated by Seki, et al. 1998, Saito, T. et al. 1999, or Naik, M. U., et al. 1999, Genbank Sequence Database (Accession No: Q9Z0F4). Applicants respectfully traverse this rejection and submit that applicants' claimed invention is not anticipated by the cited references.

According to the Office:

> "The amino acid sequence taught by Seki N. et al, 1998, Saito T et al, 1999 and Naik, MU et al, 1999 seems to be the same as the claimed mutant calcium-binding protein. . . . In the absence of evidence to the contrary, the burden is on the applicant to prove that the claimed product is different from those taught by the prior art and to establish patentable differences."

Thus it is the Office's position that the product of the sequence of the cited reference **SEEMS** to be the same as the claimed mutant calcium-binding protein of the present invention. Clearly this rejection is based on the possibility that the prior art product **MAY** inherently have the characteristics of the present invention. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing **may** result is not sufficient. (See *In re Oelrich*, 212 USPQ 323 (CCPA 1981))

The Office assumes that where there is reason to believe that an inherent characteristic may be in the prior art, the Office has the authority to require an applicant to prove that the subject matter shown in the prior art does not possess such a characteristic. However, as stated by the Board of Patent Appeals and Interferences in *Ex parte Skinner*, 2 USPQ2d 1788 (BPAI 1986), before an applicant can be put to this burdensome task, the examiner must provide some evidence or scientific reasoning to establish the reasonableness of the examiner's belief that the functional limitation is an inherent characteristic of the prior art. In the present application, the Office had provided no such evidence or reasoning to establish such a belief. As a matter of fact, the Office has repeatedly alleged that mutations and their functionality are unpredictable and without proof of the effectiveness of each mutation, enablement is lacking. Thus, the cited references are clearly lacking in enablement and as such cannot be anticipatory.

As stated by the numerous Courts, to serve as an anticipating reference, the reference must enable that which it is asserted to anticipate. "A claimed invention cannot be anticipated by a prior art reference if the allegedly anticipatory disclosures cited as prior art are not enabled." *Amgen, Inc. v. Hoechst Marion Roussel, Inc.*, 65 USPQ 2d 1385 (Fed. Cir. 2003). *See Bristol-Myers Squibb v. Ben Venue Laboratories, Inc.*, 58 USPQ2d 1508 (Fed. Cir. 2001) ("To anticipate the reference must also enable one of skill in the art to make and use the claimed invention.").

To anticipate a claim, a reference must disclose every element of the challenged claim and enable one skilled in the art to make the anticipating subject matter. The Office's speculation that the cited sequence may be anticipatory of the present invention is clearly wrong. Even pointing to the 124 amino acid residue in the cited sequence, it is very clear that this mutation is not an amine counterpart of an acidic residue. Further, there is no indication that one skilled in the art would even consider an amine counterpart replacement of the acidic residue.

Applicants submit that the terminology used by the Office when it was stated that the product of the sequences cited in the reference "**SEEMS** to be the same as the claimed mutant calcium-binding protein" (emphasis added) of the present invention indicates the Office's uncertainty as to whether the cited references are anticipatory. It is well established in the law that if a reference is ambiguous and can be interpreted so that it may or may not constitute an anticipation of an applicant's claim, an anticipation rejection under 35 U.S.C. §102 based upon the ambiguous reference is improper (*In re Hughes*, 145 USPQ 467 (CCPA 1965)). This is the current situation, because even the Office is not sure if the references are anticipatory. Accordingly, applicants request withdrawal of the §102 rejection of claims 12 and 27.

Fees Payable

Applicants have added two new independent claims and one new dependent claim, however, have also cancelled four (4) independent claims and sixteen (16) dependent claims herein. As such, no additional fee is required for entry of this amendment with the new claims. In the event a fee is required, the U.S. Patent and Trademark Office is hereby authorized to charge any additional amount necessary to the entry of this amendment, and to credit any excess payment, to Deposit Account No. 08-3284 of Intellectual Property/Technology Law.

Conclusion

Applicants have satisfied the requirements for patentability. All pending claims are free of the art and fully comply with the requirements of 35 U.S.C. §112. It therefore is requested that Examiner Davis examine the reconsider the patentability of all pending claims in light of the distinguishing remarks herein and withdraw all rejections, thereby placing the application in condition for allowance. Notice of the same is earnestly solicited. In the event that any issues remain, Examiner Davis is requested to contact the undersigned attorney at (919) 419-9350 to resolve same.

Respectfully submitted,

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